



# Mars Sample Return Planning Sterilization Working Group 2<sup>nd</sup> Meeting

Alvin Smith, Ph.D.

Lisa Pratt, Ph.D.

Brian K. Muirhead

Robert Gershman

June 10-11, 2019

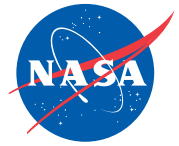
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- Follow on from the first workshop meeting in January.
- Set the stage for this meeting in terms of the following:
  - Reminder of the overall objectives for this WG
  - Review the Questions/Tasks from 1<sup>st</sup> Meeting
  - Key Outcomes from 1<sup>st</sup> Meeting
  - Objectives and agenda for this 2<sup>nd</sup> meeting
  - Reintroduce the Team

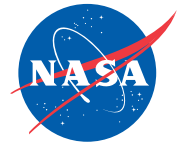
# Working Group Objectives



Mars Sample Return Pre-Formulation

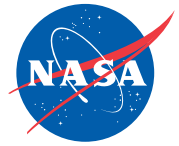
- Support the NASA Planetary Protection Officer (PPO), Lisa Pratt, in understanding what it means to sterilize Mars material and how that understanding can be applied to the Mars Sample Return (MSR) mission planning.
- Help assess methods for sterilization and identify future work needed to verify these methods
- Provide insights into the possible content of an effective plan for communicating with other agencies and the public

- NASA Policy for sample return is evolving
- Recommendations from Space Studies Board (SSB) assessment (2018):
  - A comprehensive strategic plan for managing PP policy development
  - “early consultation with....microbiologists”
  - “a means to use the best available biological and technological knowledge about back contamination and containment” That’s you.
- The assessment emphasized the need for a balance between meeting NASA’s science goals and providing the protection envisioned in NASA’s PP policy.



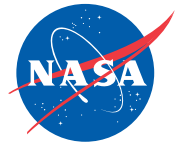
- 1) What do mean when we require “sterilization”, i.e. what is the definition of “sterilization” in the context of a threat to the Earth’s biosphere?
- 2) Can we utilize Earth micro-organisms as analogs for assessing sterilization of Mars micro-organisms? And which Earth micro-organisms?
- 3) What is the likelihood of sterility of Mars material exposed to solar UV and other space environmental factors?
- 4) At what temperature/time can “complete” sterilization be assured and what does “complete” mean?
- 5) What is the sterilizing effectiveness of cold plasma and various chemical modalities? What are synergistic benefits to combining modalities?
- 6) What would be needed to define the process for certification of a sterilization modality for backward planetary protection?

# Outcomes from 1<sup>st</sup> Meeting



- Limit our focus to biology as we know it, but consider that Mars microorganisms may be hardier to some sterilizing modalities.
- For our purposes “sterilization” means that the threat to the Earth’s biosphere posed by the subject has been reduced to an acceptably low probability. “Inactivation” of biomolecules is synonymous. We need to work on the wording so that this can be formally established.
- Heat is an acceptable modality. More work is needed to establish the temperature/time requirement. A sub-500C temperature is likely.
- Dual sterilizing modalities would be highly desirable, both for redundancy and for potential increases in effectiveness. (Maybe Heat and Chemical)
- Our tests should include microorganism biological indicators (BI) and a biomolecule BI, probably a hardy spore, a bacteriophage, and a biomolecule such as E. coli endotoxin.
- We should use sterilization techniques that are common and acceptable in industry and use industry standards in certifying our approach.
- In dealing with the public we should emphasize the improbability that anything from Mars would be pathogenic and refer to meteoritic transport from Mars to Earth.
- Recognize that industry members are enthusiastic about helping us. How can we leverage that enthusiasm?

# Tasks for the 2<sup>nd</sup> Meeting Working Group

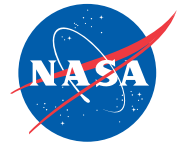


Mars Sample Return Pre-Formulation

- 1) Consensus **language for the “rationale** for backwards planetary protection” and our working group principles.
- 2) **Finalize our definition** of “sterilization” in the context of a threat to the Earth’s biosphere?
- 3) **Generate an initial list of biological indicator (BI)** organisms (Earth microbes) as analogs for assessing sterilization of Mars microorganisms?
- 4) Review new data on sterilizing **effectiveness of cold plasma**.
- 5) Understand the likelihood of **UV on sterilizing** of Mars material exposed to UV in flight, solar UV, other space environmental factors? Discuss experiments to address.
- 6) Review new data on **temperature/time** for selected BIs for assessing “complete” sterilization. Decide on next experiments to address data gaps.
- 7) Understand **engineering challenges** for implementing a modality in flight.
- 8) Discuss and **design future experiments** to understand the data gaps and synergistic effects of a dual modality approach to sterilization.

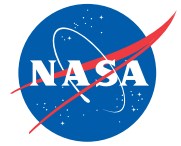
- Consensus on a design of experiments to fill current data gaps in sterilization
- A publishable manuscript that addresses key sterilization questions that would be referencable in official documents.
  - Published meeting report authored by external SME with support from JPL
  - Individual publications by working group members (experimental data)
- We are also seeking help in explaining the nature of any potential threat from returning Martian material, and our approaches to dealing with it effectively, with public and stakeholders.
  - Effectiveness of sterilization modalities
  - Analogies to the nature, scope and mitigation techniques of potentially comparable threats (e.g. Flu) currently and in the past
  - Rationale for Backwards PP – Group “Vision” statement

# Agenda Day 1



MSR Planning Sterilization Working Group 2nd Meeting Agenda // June 10-11, 2019 // Johnson & Johnson Sterility Assurance, Raritan, NJ				
Day 1			start	minutes
1	Room Opens		7:30 AM	30
2	Meeting Opening/Introduction	Smith	8:00 AM	15
3	Member Introductions	All	8:15 AM	15
4	MSR Program Status	Muirhead	8:30 AM	15
5	Sample Safety Assessment Protocol (SSAP) WG Overview and Status	Pratt	8:45 AM	15
6	Review of WG Action Item Status	Smith	9:00 AM	30
7	Introduction to Rationale for Backward Planetary Protection and Sterilization Working Group Principles	Gershman	9:30 AM	30
BREAK			10:00 AM	10
8	Definition of Sterilization (Industry Standards)	Winters/O'Hara	10:10 AM	45
8A	Discussion	All	10:55 AM	15
9	Biological Indicators for Testing			
9A	Understanding Earth Analogs	Stricker	11:10 AM	20
9C	Identifying Indicator Organisms	Benardini	11:30 AM	20
9D	Discussion	All	11:50 AM	40
Lunch			12:30 PM	60
10	Proteinacious Molecule tests (Preliminary data and Future Study Design)	Seto/Schubert	1:30 PM	30
11	Other Sterilization Modalities - Plasma, Chemical (Preliminary data and Future Study Design)	Stricker/Logar	2:00 PM	30
11A	Discussion	All	2:30 PM	60
BREAK			3:30 PM	10
12	UV Sterilization			
12A	UV + Heat + Vacuum Biocidal Conditions in Space for the Mars2020, MSR Rover/Ascent, and ERO Spacecraft	Schuerger	3:40 PM	20
12B	Preliminary UV findings	Shirey/Coohill	4:00 PM	30
12C	Discussion	All	4:30 PM	30
Adjourn			5:00 PM	
13	Dinner	All	7:00 PM	~

# Agenda Day 2



Day 2			start	minutes
14	Room Opens		7:30 AM	30
15	Temperature/time, D-values, Future tests, and Margin Required (Historical Data and Future Study Design)	Schubert/Mitchell	8:00 AM	45
16	Implementing Sterilization in Flight	Hendry	8:45 AM	30
17	Testing of JPL microbes at J&J (Future Study Design)	Smith/Logar	9:15 AM	30
17A	Discussion	All	9:45 AM	30
BREAK			10:15 AM	10
18	Rationale for Backward Planetary Protection and Sterilization Working Group Principles	Gershman/All	10:25 AM	30
19	First Meeting Report - Status update and tuning for release	Winters/Craven/Smith	10:55 AM	15
20	Meeting Publications/Presentations	Smith	11:10 AM	15
21	Outstanding Assignments/Timeline Review/Next Meeting	Muirhead/Smith	11:25 AM	20
22	Closing Remarks	Muirhead/Pratt/Smith	11:45 AM	15
Adjourn			12:00 PM	~
23	Tour of J&J Facilities	All	12:30 PM	90
Lunch/Depart			2:00 PM	60

# WG External SME Membership

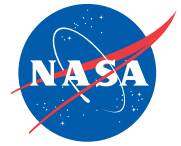


Mars Sample Return Pre-Formulation

## Invited Subject Matter Experts (SMEs)

Name	Discipline	Affiliation	Email
John Logar	Sterilization mathematician, broad sterilization expertise	Johnson & Johnson	jlogar8@its.jnj.com
Rohit Chitale, MPH, Ph.D.	Epidemiologist, Public Health	PATH and Defense Advanced Research Projects Agency (DARPA); (Experience with Centers for Disease Control and Prevention)	rchitale@gmail.com
Thomas Coohill, Ph.D.	UV Sterilization/BioPhysics	Department of Physics and Astronomy; Siena College Loudonville, NY	tcoohill@siena.edu
Emily Craven, P. Eng	Physicist and engineer, radiation dosimetry (gamma and electron beam)	Mevex	ecraven@mevex.com
Paul Littley	Ethylene Oxide and Water Systems Expert	Nelson Labs	plittley@nelsonlabs.com
Rocco Mancinelli, Ph.D.	Astrobiology and Microbiology	NASA-Ames; Bay Area Environmental Research Institute	Rocco.L.Mancinelli@nasa.gov
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Kevin O'Hara	Physicist, radiation dosimetry (gamma and electron beam), Mathematical modeling	Sterigenics	kohara@sterigenics.com
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Martell Winters	Microbiology, Radiation Sterilization	Nelson Labs	mwinters@nelsonlabs.com
Kris Zacny, Ph.D.	Planetary drill developments, sterilization protocols	Honeybee Robotics Spacecraft Mechanisms Corporation	KAZacny@honeybeerobotics.com
Peter Emanuel, Ph.D.	Senior Research Scientist (ST) for Biotechnology	U.S. Army Edgewood Chemical and Biological Center	peter.a.emanuel.civ@mail.mil
Andrew C. Schuerger, Ph.D.	Microbiology and Astrobiology	University of Florida Space Life Sciences Lab	schuerg@ufl.edu

# WG NASA/JPL and ESA Participation



Mars Sample Return Pre-Formulation

## NASA and JPL Participants

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Charles Budney	Mars Sample Return Systems Engineer	<a href="mailto:Charles.J.Budney@jpl.nasa.gov">Charles.J.Budney@jpl.nasa.gov</a>
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Erin Lalime, Ph.D.	NASA-GSFC Aerospace Engineer / Planetary Protection Engineer	<a href="mailto:erin.lalime@nasa.gov">erin.lalime@nasa.gov</a>
Aaron B. Regberg, Ph.D.	NASA-JSC Geomicrobiologist, Astromaterials Acquisition & Curation Office	<a href="mailto:aaron.b.regberg@nasa.gov">aaron.b.regberg@nasa.gov</a>



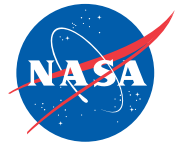
# 6. Review of WG Action Item Status

Alvin L. Smith, Ph.D., PMP

June 10, 2019

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# Action Items



Action Item	Assigned	Due Date
● Write a publishable meeting report.	M. Winters/E. Craven/A. Smith	3/30/19 - Initial Draft
● Possible sterilization testing of JPL clean room microbes at J&J, JPL/Logar, when?	J. Logar/L. Pratt/B. Muirhead/A. Smith	4/1/19
● 2A. Understand what sterilization techniques they are currently using.	A. Smith/J. Logar/N. Benardini	-
● 2B. Work any legal paperwork (MOU, NDAs, NASA Space Act).	Muirhead/Pratt	-
● 2C. What are some potential projects we need to answer data gaps?	JPL Sterilization Team	-
● Develop strategy of microbial reduction using current BI's but demonstrating performance against a more likely set of microbes (e.g. most common, E. Coli, ), JPL, when? (e.g. 10 things that we believe we need to protect against that we could handle safely.)	W. Schubert/B. Shirey/M. Stricker/G. Ruvkun	5/1/19
● Prepare discussion on general probabilistic argument for safety of bio-sphere starting at Mars, JPL, when?	B. Gershman	4/1/19
● Review AAMI for industry definitions and tailor for definitions of "sterilization," inactivation, etc. for MSR needs but recognizing any possible issues for industry, JPL/Winters, when?	M. Winters, K. O'Hara	4/1/19
● Does possible Mars material meet the criteria for containment in BSL-4 facility or beyond?	R. Chitale/A. Smith	4/1/19
● Agenda for next meeting (Policy decisions for MSR. (e.g. Heat temp as used by industry standards.)	B. Muirhead/B. Gershman	4/1/19
● Further analysis and discussions on UV sterilization (Create sub group to investigate UV. Push this to the "Dust" group.	B. Shirey/M. Hendry/T. Coohill	-
● How and when do we develop a D-value representation, including the BI, that can be certified by OPP that we can design to in the 200-300 C (TBC). How to margin the time or temperature? JPL, when?	G. Mitchell/M. Winters/W. Schubert	5/1/19
● Update system engineering of different BTC/sterilization modalities and consider where we could apply the techniques to make our job easier, cheaper,	M. Hendry/J. Umland/B. Gershman	4/1/19
● 10A. Provide Lisa with data from WG to make policy decisions (e.g. BI, temp., D-value, etc... to lower temp from 500C.)		5/1/19
● Add a chemical expert to the WG	A. Smith	5/1/19



# 17. Testing of JPL microbes at J&J (Future Study Design)

Alvin Smith and John Logar

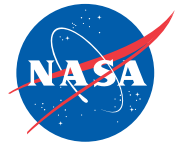
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## How do we certify our sterilization process data and get to regulatory consensus?

- We will leverage FDA Testing Guidelines for Medical Devices – *“Submission and Review of Sterility Information in Premarket Notification (510(k)) Submissions for Devices Labeled as Sterile Guidance for Industry and Food and Drug Administration Staff”* – January 21, 2016
- Our proposed sterilization modalities are **Established and Novel** based on these guidances (e.g. dry heat, UV)
- Ensure our research testing and data met minimum standards for acceptance for either Established or Novel techniques based on FDA guidances.

# Methodology Testing: Established vs. Novel (Where do we fit?)



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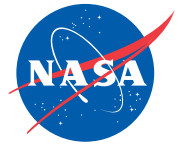
- **Established Testing**

- For the sterilization method, the sponsor should provide the following:
- Description of the sterilization method (data not needed at submission)
- Description of the sterilization chamber if not rigid, fixed
- Sterilization site
- In the case of radiation sterilization, the radiation dose
- For chemical sterilants (e.g., EO, H<sub>2</sub>O<sub>2</sub>), the maximum levels of sterilant residuals that remain on the device
- Description of the method used to validate the sterilization cycle
- State the sterility assurance level (SAL) of 10<sup>-3</sup> – 10<sup>-6</sup>
- Pyrogenicity testing (assess bacterial endotoxins)

- **Novel Testing**

- All of the above...
- Comprehensive description of the sterilization process;
- Method used to validate the sterilization cycle (e.g., the half-cycle method);
- Validation protocol
- Sterilization validation data (Include scientific literature).

# How do we get there from here?



- Ensure our research and data meet minimum standards for acceptance for either Established or Novel techniques based on FDA guidances.
  - Proper positive and negative controls (where applicable). Test organisms.
    - Biological Indicators
    - Analogs
      - Microbe characterization
  - Testing methodologies should adhere to proven standards
    - Spore test; D-Values; fraction negative; Spearman-Kärber, etc...
    - V&V of Equipment (ISO standards)
  - Data storage and maintenance (e.g. industry standards?)
    - Electronic databases
    - Access
  - Peer-reviewed data (publications, conferences, etc...)
  - Transparency across all regulatory agencies/stakeholders (DoD, CDC, FDA, USDA, NASA). Proposed process is clear and rooted in good science.
  - Data should become a part of the Risk assessments

- Following 1<sup>st</sup> meeting John Logar (J&J), offered to assist in closing sterilization data gaps.
- J&J has long history in delivering sterile products through the development and use of numerous terminal sterilization technologies here at Research & Development facility located in New Jersey.
- Initial conversation began with NASA-PPO to understand scope and capabilities available at J&J
- Ongoing discussions with J&J, NASA-JPL, and NASA-HQ on contract vehicles.
- Provide a design of experiments (DOE) to support sterilization of NASA-JPL supplied BI's at J&J and potentially other partners

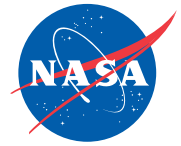
# Sterilization Modalities



- Comparison based on current capabilities and overall data history

JPL Sterilization Modalities	J&J Sterilization Modalities
<b>Heat</b> <ul style="list-style-type: none"><li>• Dry Heat Oven</li><li>• Steam Autoclave</li></ul>	<b>Heat</b> <ul style="list-style-type: none"><li>• Dry Heat Oven<ul style="list-style-type: none"><li>• 311 liters (11ft<sup>3</sup>) chamber capable of up to 360°C</li></ul></li><li>• Steam Autoclave</li></ul>
<b>Radiation Sterilization</b> <ul style="list-style-type: none"><li>• Gamma</li><li>• E-beam Irradiation</li></ul>	<b>Radiation Sterilization</b> <ul style="list-style-type: none"><li>• Gamma</li><li>• E-beam Irradiation</li></ul>
<b>Gas Sterilization</b> <ul style="list-style-type: none"><li>• Ethylene Oxide</li><li>• Vapor Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>)</li></ul>	<b>Gas Sterilization</b> <ul style="list-style-type: none"><li>• Ethylene Oxide<ul style="list-style-type: none"><li>• 75ft<sup>3</sup> 100% EO Chambers</li><li>• 5ft<sup>3</sup> 100% EO Chamber</li></ul></li><li>• Noxilizer (NO<sub>2</sub>)<ul style="list-style-type: none"><li>• 12.7ft<sup>3</sup> chambers</li><li>• Room temperature</li></ul></li><li>• STERRAD Vapor Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>)</li><li>• Others? Chloride Dioxide?</li></ul>

# Considerations for Testing



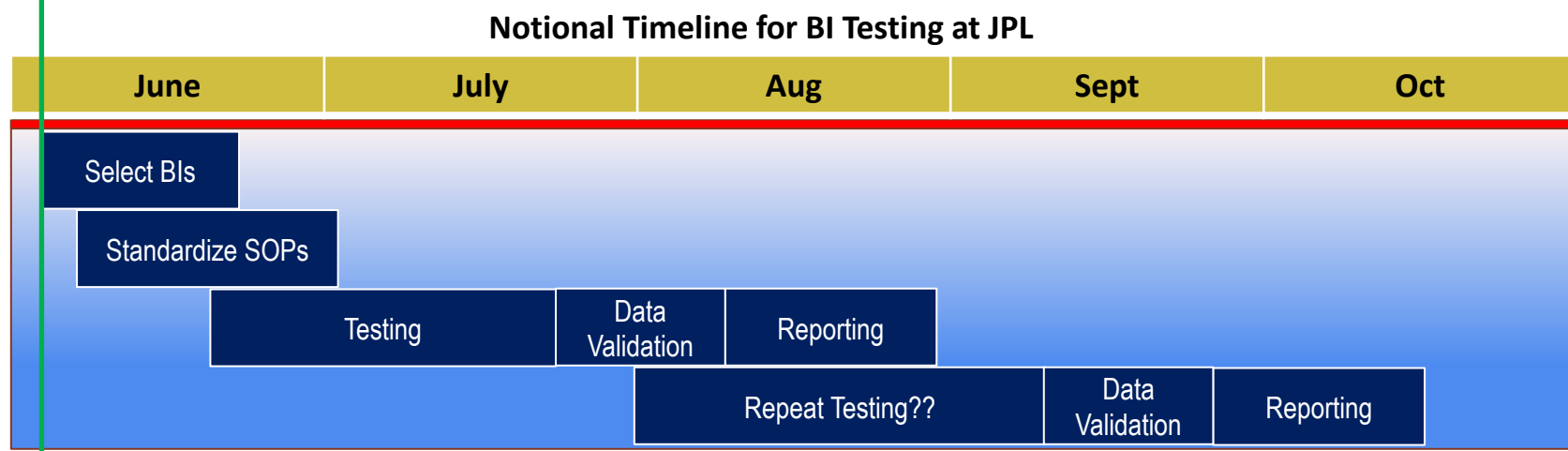
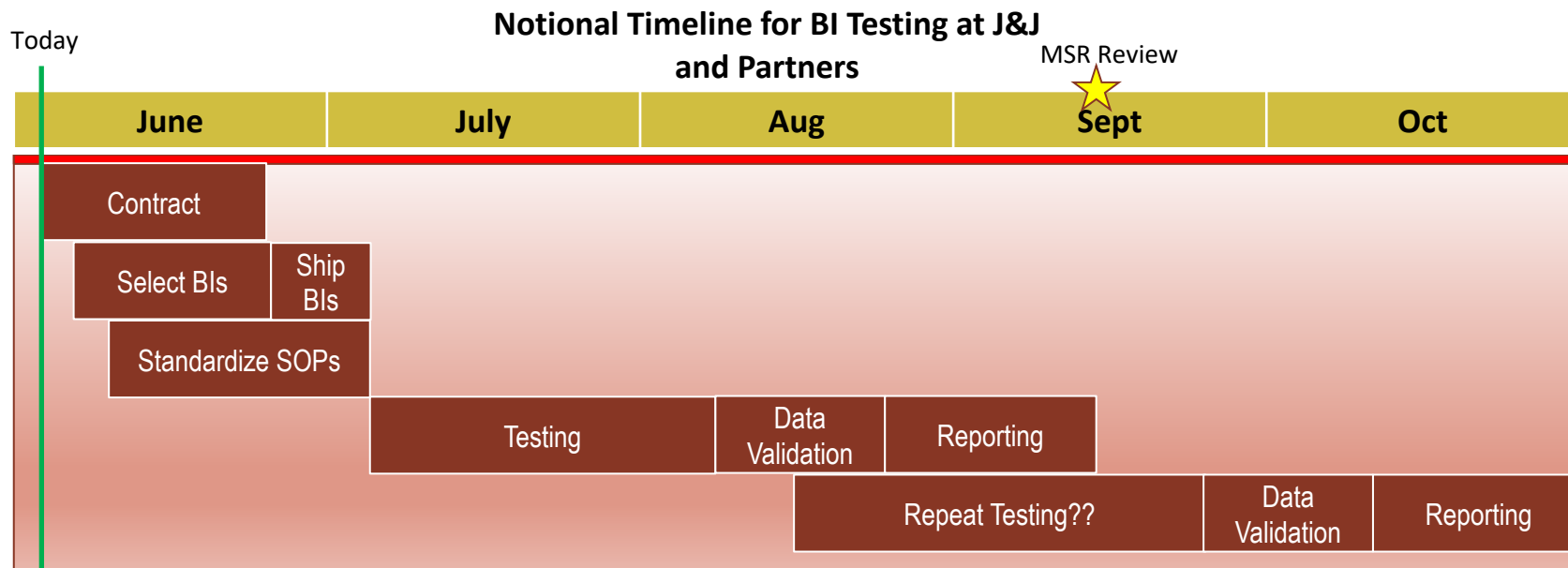
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- BI Testing
  - Individually or in a mixture?
  - Desiccated on coupon or in solution (e.g. culture broth)?
  - Add Martian regolith simulant or pure?
- SOP Standardization
  - Can we choose ISO-like? (e.g. ISO 111138, ISO 17025 – Test/Calibration Labs)
  - Qualification – FDA standards for Medical Devices?
  - V&V Equipment?
- Test Procedures
  - Individual sterilization modalities
  - Dual modalities; Synergistic effects
  - Testing in parallel
- Reporting
  - Software – General Principles of Software Validation; Final Guidance for Industry and FDA Staff” – Jan 11, 2001. Validated according to “state-of-the-art” (e.g. lifecycle development, risk management, and V&V)
  - Formatting

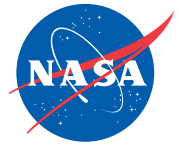
# Notional Timeline (3-4 Months)



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# Next Steps (Short and Long Term)



Mars Sample Return Pre-Formulation

## 3-4 Months

- Formalize the Agreement (e.g. contract, SAA, PO, etc...) with partners
- Arrange meeting next couple weeks:
  - Offline discussions with JPL SMEs, J&J SMEs, Academic SMEs
    - Finalize Initial BIs (make decisions on testing matrices)
    - Share and standardize testing SOP (industry help)
    - Decide on realistic timelines and milestones
    - Discuss results (Iterate on testing and data analysis)
- Report data back to Group (virtually)
- Determine Path Forward (more testing, make programmatic decisions)
- Third Workshop??
  - Focused on data, down-selection, and engineering

**Long Term:** Possibly Release RFIs or NASA ROSES Data Call